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BRIEF COMMUNICATION

Biochemical and Behavioral Effects of Lesions of Raphe Nuclei in Aggressive Mice

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KOSTOWSKI, W. AND L. VALZELLI. *Biochemical and behavioral effects of lesions of raphe nuclei in aggressive mice.* PHARMAC. BIOCHEM. BEHAV. 2(2) 277–280, 1974. – A decrease of brain 5-hydroxytryptamine turnover was described to be present in mice made aggressive by prolonged isolation. The lesion of brain serotoninergic pathways decreases both 5-hydroxytryptamine and 5-hydroxyindolacetic acid levels in the forebrain and completely abolishes the aggressive behavior in previously fully aggressive mice. No change of aggressive behavior or of serotonin levels was observed in aggressive mice submitted to lesions of other areas.

Raphe nuclei Lesions Isolation-induced aggressiveness Mice Serotonin 5-Hydroxyindolacetic acid

AGGRESSIVE behavior resulting from prolonged isolation both in male mice and rats of some particular strains, i.e. Albino Swiss mice and Wistar rats, was reported to be accompanied by a decreased turnover of brain 5-hydroxytryptamine (5-HT) [4, 14, 15, 16, 18, 19]. Clearcut evidence of a direct relationship between the alteration in brain 5-HT turnover and the maintenance of aggressiveness by isolation is not yet available, although many experimental findings [17] support the hypothesis of the involvement of this neurochemical mediator in the regulation of the aggressive behavior.

It was recently reported by Di Chiara *et al.* [3] that pharmacological manipulations of brain 5-HT metabolism can modify emotional and aggressive behavior of laboratory animals. They found that isolated but non-aggressive rats, showing only a poor or no mouse-killing activity, become muricidal when pretreated with p-chlorophenylalanine, a widely known inhibitor of brain 5-HT synthesis [8]. Moreover, lysergic acid diethylamide (LSD_{25}), which was described to be an antagonist of brain 5-HT [1], was reported to induce aggressiveness in normal animals [6] and to decrease the aggressive behavior evoked by painful stimulation or prolonged isolation [13].

Stereotaxic lesions of the midbrain raphe serotoninergic system seem to be a useful method for producing a selective depletion of brain 5-HT as a consequence of the degeneration of the forebrain serotoninergic projections [7,9]. It is, therefore, possible that such lesions can provide useful information regarding the relationship between 5-HT and aggression.

The present communication deals with an attempt to study both the behavioral and biochemical effects produced by lesions of the raphe system in isolated-aggressive mice, providing some preliminary results and some methodological approaches in this field.

MATERIALS AND METHOD

Male Albino Swiss mice, kept singly housed in Makrolon cages for four weeks in order to induce aggressive behavior [15] were used. The animals, weighing 33 ± 2 g at the beginning of the surgical intervention, were maintained in a controlled environment (22°C ± 1 and 60% relative humidity), with food and water ad lib and in a preset dark–light cycle (12 hr light and 12 hr dark). Since no atlas showing the stereotaxic parameters of the brain raphe area of mice is yet available, we calculated the necessary coordinates, as reported in Fig. 1, using, in part, the Atlas by Sidman *et al.* [12] and the one of the mouse diencephalon by Montemurro and Dukelow [11].

The stereotaxic parameters employed were the following: P = 0.3 to 0.5 mm (behind the interauricular line); H = 2.0 to 2.1 mm (above the interauricular line) and L = 0.0 mm; the electrode was inserted with a lateral inclination of 10° to avoid the sagittal sinus. The upper incisor bar was 1 mm above the interauricular line and the A-P distance between the upper incisor bar and the interauricular line was 13.9 mm.

Animals were placed, under light ether anesthesia, on a Stoelting Stereotaxic Instrument for the rat provided with a

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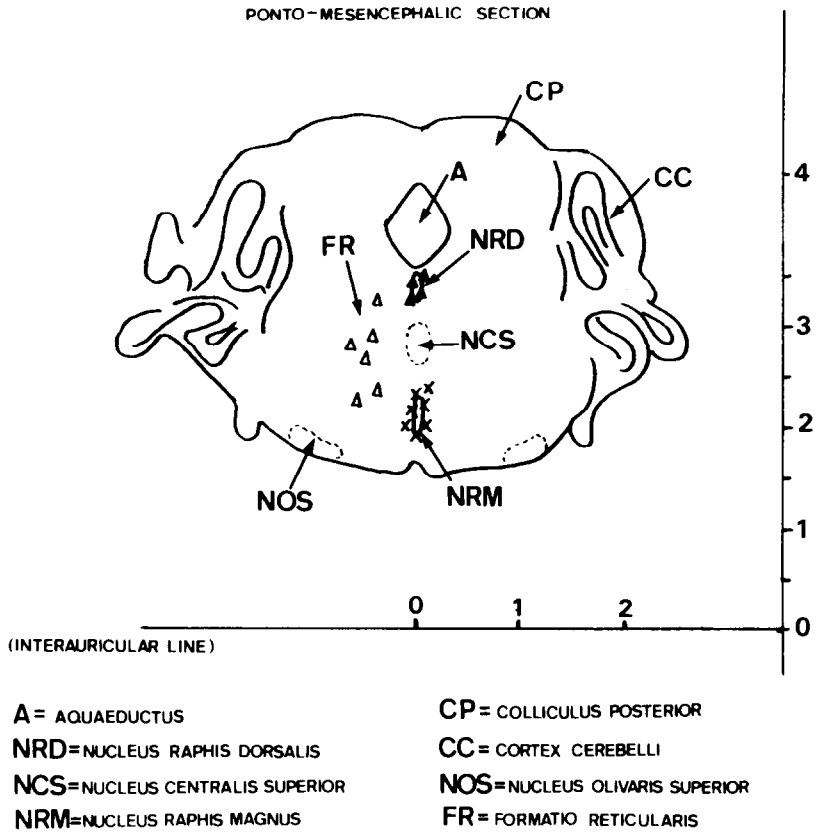

FIG. 1. Sites of lesions made in the pontine portion of the raphe system in mouse brain. The section corresponds to plates No. 430 and 431 of the atlas of Sidman *et al.* [12]. (xxx = lesions situated within the area of the nucl. raphis magnus; ▲ = lesions of dorsal raphe; △ = lateral lesions. The symbols indicate the position of the electrode).

special head holder prepared for mice. Lesions were made through an insulated stainless steel electrode (dia. 0.2 mm) with an 0.5 mm tip scraped free of insulation and with a direct anodal current of 0.6–0.8 mA applied for 6 to 10 sec. The animals completely recovered from surgery within 24 hr.

Some animals were randomly selected for histological examination to determine the exact location of the lesions performed. The lesions were found to be in the ventral part of the raphe, at the pontine and pontine-mesencephalic levels, in the area corresponding to the nucleus magnus of the raphe according to Sidman *et al.* [12]. In some other animals, the lesion was shifted laterally in such a way to involve the pontine and mesencephalic part of the reticular formation.

Trials of lesioning the dorsal raphe area resulted in a high level of postoperative lethality (60–70% of animals died during the first week following the surgical intervention) so that such experiments were not continued.

Sham lesioned animals were submitted to the full surgical procedure except that the current was not delivered through the electrode lowered into the brain.

The aggressive behavior was scored according to Valzelli [15] while the attack and defence attitudes was checked either by putting in contact one previously isolated-aggressive and lesioned mouse with two normally-housed and non-aggressive mice, or testing one previously isolated-aggressive and lesioned mouse with another isolated-aggressive but intact mouse. Such behavioral observations were made during a period of 5 min of contact (Fig. 2).

The determinations of the forebrain 5-HT and 5-hydroxyindolacetic acid (5-HIAA) levels were made according to Giacalone and Valzelli [5] and the determinations of norepinephrine (NE) and dopamine (DA) were performed according to Chang [2] and Laverty and Taylor [10].

RESULTS AND DISCUSSION

As described elsewhere for the rats [9], lesions of the ventral raphe nuclei also result in an enduring motor hyperactivity and an increased excitability to environmental stimuli in mice. Animals in which the lesion was made laterally showed only an occasional and transient increase of motor activity and rotatory movements. As shown both in Fig. 2 and in Table 1 the lesion of the ventral raphe area completely abolishes the aggressive behavior in previously fully aggressive mice as well as the interest toward their non-aggressive partners; moreover, such lesioned animals show no tendency to attack, a very poor defence, a general hyperexcitability and an escape behavior when tested against other aggressive mice.

Lateral lesions decrease only to a slight extent the

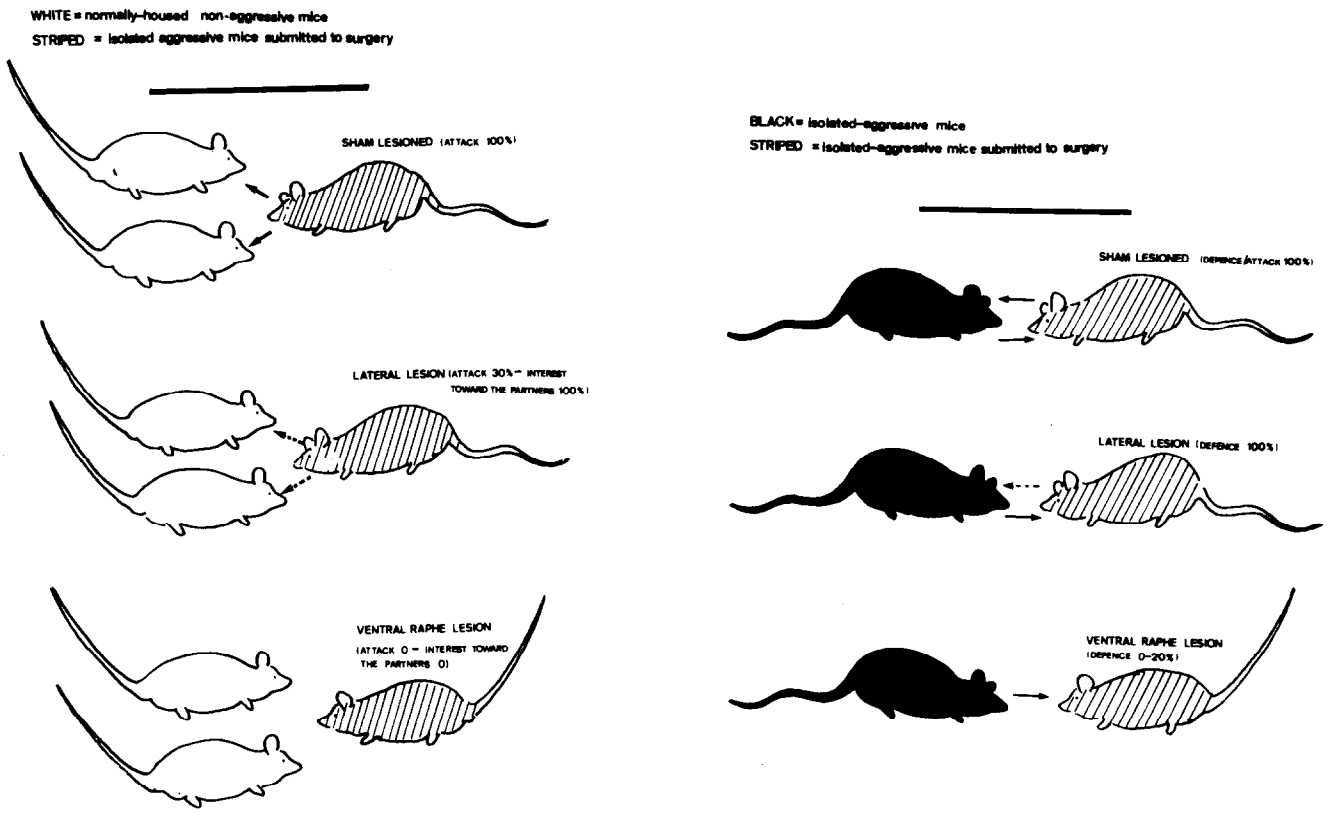

FIG. 2. Behavioral patterns shown by isolated-aggressive mice submitted to lesions toward normal or non-lesioned isolated-aggressive mice.

TABLE 1

EFFECT OF VENTRAL RAPHE NUCLEI (VR) OR LATERAL NUCLEI (L) LESIONS ON BRAIN 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLACETIC ACID (5-HIAA) LEVELS AND ON SOME BEHAVIORAL PATTERNS OF ISOLATED-AGGRESSIVE MICE

		Brain levels (μg/g ± S.E.)*		Behavioral patterns				
					% of animals showing:			
Treatment	Days after surgery	5-HT	5-HIAA	Intensity of aggressive behavior†	Interest toward partners	Attack	Defence	No. of animals per group
Sham operated	3–4	0.64 ± 0.03	0.32 ± 0.02	100	100	83	100	12
	7–9	0.67 ± 0.02	0.27 ± 0.03	100	100	90	100	10
VR lesion	3–4	0.38 ± 0.02‡	0.17 ± 0.01‡	0	0	0	25	8
	7–9	0.32 ± 0.02‡	0.17 ± 0.01§	0–25	0	0	50	6
L lesion	3–4	0.66 ± 0.02	0.29 ± 0.02	50–75	100	75	100	8
	7–9	0.65 ± 0.02	0.28 ± 0.02	75–100	100	87	100	8

*The figures correspond to the mean of 4–6 determinations.
†According to the score elsewhere reported [15]
‡$p<0.001$ in respect to sham-operated
§$p<0.005$ in respect to sham-operated

aggressiveness, so that at least 70% of such mice show attacks when tested both with other aggressive mice or with other non-aggressive partners. Finally, sham-lesioned mice are normally aggressive and perform attacks at an 80% level toward non-aggressive animals.

From the biochemical point of view, while no significant changes of the forebrain 5-HT and 5-HIAA are induced by the lesions of the lateral pontine-mesencephalic area, lesions of ventral raphe result in a marked decrease of both these biochemical parameters (Table 1), without any modification of the brain levels of NE or DA (NE and DA in forebrain of sham-lesioned mice = respectively 0.50 ± 0.02 and 2.89 ± 0.19 μg/g; NE and DA in forebrain of ventral raphe-lesioned mice = respectively 0.48 ± 0.02 and 2.12 ± 0.19 μg/g.

These data indicate that lesions of the ventral nuclei of the midbrain raphe in mice, made aggressive by prolonged isolation, suppress the aggressive behavior. Moreover, this effect seems to be strictly related to the decrease in the forebrain 5-HT and 5-HIAA level, since no change or only a very slight decrease in the aggressiveness was observed in mice subjected to lesions of the lateral nuclei of the pontine-midbrain area. These findings agree with those of other authors showing that drugs blocking 5-HT synthesis or receptors might decrease the isolation-aggressiveness in mice [20]. The results may appear somewhat contradictory to the reports that p-chlorophenylalanine potentiates the muricidal behavior in rats [3] or that aggressiveness by isolation in mice is accompanied by a decrease of brain 5-HT synthesis [4,15]. However, no direct comparison can be made between such data and the results reported here because the muricidal behavior shown by rats should be considered something different from the aggressiveness induced in mice by isolation and also because both the previously quoted findings refer to the development of the aggressive behavior and not, as the experiments reported here, to mice already made aggressive by isolation.

The data reported here underline the importance of brain 5-HT and of serotoninergic pathways in the expression of aggressive behavior by isolation in mice.

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